Effects of *Carpobrotus edulis* invasion on soil gross N fluxes in rocky coastal habitats

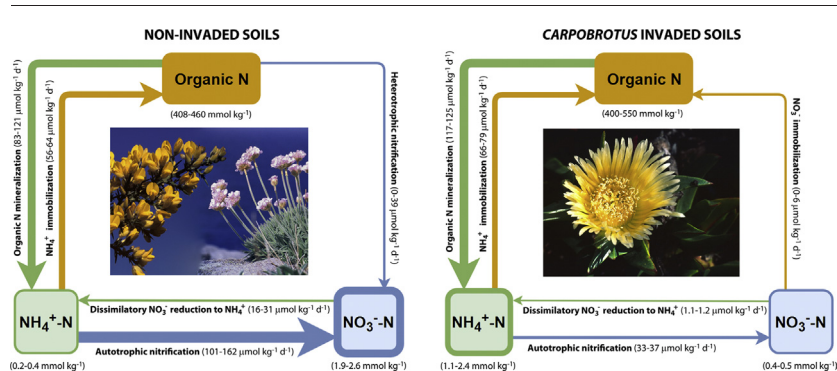Cristina Vieites-Blanco [a,b], Serafín J. González-Prieto [a,*]

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HIGHLIGHTS

- Gross N fluxes in *C. edulis* invaded and uninvaded soils were modelled with *Ntrace*.
- *Carpobrotus edulis* invasion affected gross N fluxes in the 0–5 and 5–10 cm layers.
- Invasion reduced NO_3^- producing and consuming rates and raised NH_4^+ immobilization.
- Invaded soils showed lower net nitrification, N mineralization and N availability.
- D_{NRA} was usually the exclusive/dominant NO_3^- consumption rate in our C-rich soils.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

The effects of alien plants on whole nutrient cycles have been scarcely studied, despite the increasing evidence on their impact on nutrient pools and fluxes. *Carpobrotus edulis*, a dangerous invasive plant in coastal areas worldwide, is considered an ecosystems engineer which, by changing many soil properties, benefits its own invasion and hampers the restoration of the invaded habitats. To study, for the first time, the 'true' impact of *C. edulis* on the soil N cycle, we used a paired ^{15}N labelling experiment and a *Ntrace* compartment model to estimate the gross N fluxes in the 0–5 cm and 5–10 cm soil layers of non-invaded and *C. edulis* invaded areas of two temperate-humid coastal rocky locations. *Carpobrotus edulis* invasion generally increased NH_4^+ immobilization (I_{NH4}, 1.19–4.48×), presumably due to a lower N availability for the microbiota. The invasion also decreased autotrophic nitrification (O_{NH4}, 0.20–0.79×), either by a direct effect over soil microbiota or by the acidification triggered by *C. edulis*. Unexpectedly, the dissimilatory nitrate reduction (D_{NRA}) was the exclusive NO_3^- consuming process modelled on most of the studied soils, although the incubation was aerobic. Apparently, the high organic C content of these soils induced a higher O_2 consumption and the formation of anaerobic microsites where the D_{NRA} could have taken place. The lower NO_3^- availability of invaded soils could explain their lower D_{NRA} rates (0.04–0.70×) compared to native soils. Both D_{NRA} and O_{NH4} were more affected in the 0–5 cm layer, but the invasion also significantly affected N rates in the 5–10 cm layer. Overall, net nitrification and mineralization generally decreased in the invaded soils. This study shows that the invasion of *C. edulis* alters soil gross and net N fluxes in a 0–10 cm depth through its effects on soil properties and microbiota.

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1. Introduction

Plant invasions can impact the base of all terrestrial ecosystems - the soil - by modifying its physical and chemical characteristics, nutrient cycling and microbiota (Dassonville et al., 2008; Ehrenfeld, 2003; Ehrenfeld and Scott, 2001; Suding et al., 2013; Vanderhoeven et al., 2005; Weidenhamer and Callaway, 2010). Of particular importance are the invaders' effects on the N cycle, as N is the most widespread limiting nutrient in ecosystems (Galloway et al., 2004; Vitousek and Howarth, 1991) and N availability can affect plant community structure. Therefore, changes on soil N pool, N residence time and ecosystem N cycling feedbacks can provide a competitive advantage of invaders over natives, facilitating the invasion (Laungani and Knops, 2009; Theoharides and Dukes, 2007).

Invasive plants can modify the soil N cycle through their impacts on soil microbial communities, litter decomposition and soil properties (Laughlin, 2011; Schaeffer et al., 2012; Wang et al., 2015). These changes can be driven by differences in phenology, leaf traits, plant litter composition, N use, N residence time, interaction with herbivores, symbiosis with native or co-introduced N_2-fixing bacteria, effects on soil microbiota structure and activity, and effects on the microclimate (Castro-Díez et al., 2014; Corbin and D'Antonio, 2004; Knops et al., 2002; Laughlin, 2011; Laungani and Knops, 2009; Lee et al., 2017; Mack and D'Antonio, 2003). The modifications of the N cycle can be influenced by the characteristics of the invaded site and the invasive species, being often stronger in mild climates and islands (Castro-Díez et al., 2014) and when the traits of the invasive plant differ from those of the native flora (Castro-Díez et al., 2014; Lee et al., 2017).

The effects of invasive plants on the soil N cycle are species and context-dependent (Wang et al., 2015), but usually involve increases on the N pools and fluxes (Lee et al., 2017). Generally, a positive plant-soil feedback - higher litter quality of the invader resulting in higher decomposition rate and N availability - is found (Knops et al., 2002; Liao et al., 2008; Stark and Norton, 2015; Wang et al., 2015).

Furthermore, impacts of invasive plants on the soil N cycle can persist after their removal (Elgersma et al., 2011). This is particularly true when invasions involve changes in microbial communities (Elgersma et al., 2011) or in N stock and availability, affecting the recolonization patterns and the restoration of the ecosystem (Corbin and D'Antonio, 2004), and increasing the risk of secondary invasions by ruderal nitrophilous plants (Novoa et al., 2013; Santoro et al., 2012; Santoro et al., 2011). As net N fluxes are the result of several counteracting processes (Murphy et al., 2003), they do not adequately reflect the impacts of invasive plants (Piper et al., 2015). Therefore, studies on the effect of invasive plants on the gross N fluxes are necessary to fully understand how the invasion is disturbing the N cycle. However, these studies are scarce and mostly focussed on annual grasses (Booth et al., 2003; Hawkes et al., 2005; Parker and Schimel, 2010; Piper et al., 2015; Schaeffer et al., 2012; Stark and Norton, 2015), with only a few exceptions for perennial grasses (Thorpe and Callaway, 2011), leguminous plants and trees (Laungani and Knops, 2012).

Carpobrotus edulis (L.) N.E.Br is a perennial mat-forming succulent plant from the Aizoaceae family (Campos et al., 2004). Although it is original from South Africa, it has been introduced worldwide with ornamental, medical and land stabilisation purposes (Campos et al., 2004; Malan and Notten, 2006; Maltez-Mouro et al., 2010). Currently, it is considered a highly invasive plant outside its native range (Global-Invasive-Species-Database, 2016; Vilà et al., 2006); the same is true for its hybrid *Carpobrotus aff. acinaciformis* (L.) L. Bolus.

It has been repeatedly proved that *C. edulis* affects the physicochemical properties (Conser and Connor, 2009; Novoa et al., 2013; Novoa et al., 2014; Vieites-Blanco and González-Prieto, 2017; Vilà et al., 2006) and microbiota (de la Peña et al., 2010) of the invaded soil. Through its effects on soil and other abiotic elements, *C. edulis* favours its own invasion (Conser and Connor, 2009), and the establishment of opportunistic native species as well (Novoa et al., 2013), so it is considered an

ecosystem engineer (Conser and Connor, 2009; Molinari et al., 2007). This alien invasive plant can increase (Badalamenti et al., 2016; Santoro et al., 2011) or decrease (Conser and Connor, 2009; Vieites-Blanco and González-Prieto, 2017) soil total N, and increase (Novoa et al., 2014) or decrease (Novoa et al., 2014; Vieites-Blanco and González-Prieto, 2017) soil NH_4^+ and NO_3^- availability. This variability on the magnitude and direction of the impacts of *C. edulis* on the invaded soil suggests a context-dependency of its effects (Vieites-Blanco and González-Prieto, 2017), also seen for other invaders (Vilà et al., 2011). Moreover, its necromass has a higher C/N ratio than native necromass (Badalamenti et al., 2016; Vieites-Blanco and González-Prieto, 2017). The impact of *C. edulis* on gross N fluxes has not been studied yet, despite having this alien invasive species site-dependent effects over N compounds (Novoa et al., 2013; Novoa et al., 2014), and being still controversial the interpretation of the processes involved in these changes.

Therefore, the aim of the present study was to evaluate, for the first time, the effect of *C. edulis* invasion on gross N rates. We used a paired ^{15}N labelling experiment and the state-of-the-art *Ntrace* compartment model (Müller et al., 2007) to estimate the gross N fluxes in the 0–5 cm and 5–10 cm soil layers (the most affected by alien roots and necromass) of non-invaded and *C. edulis* invaded areas in two temperate-humid rocky areas (NW Spain shoreline).

2. Material and methods

2.1. Site and sampling description

Two representative study sites of the coastal rocky areas invaded by *C. edulis* were selected in NW Spain: Punta Nariga (43°19′13″ N, 8°54′34″ W; recently invaded: 15–20 years) and Sálvora Island (42°27′55″ N, 9°0′49″ W; long-term invasion: ~80 years); both of them with Umbric leptosol soils (IUSS Working Group, 2014) formed over granitoid rocks. Climate is characterised by a mean annual temperature of 14–16 °C and a mean annual precipitation of 1400–1800 mm (AEMET-IMP, 2011). The distance to the sea was similar in both sites: 70–80 m in Punta Nariga and 65–95 m in Sálvora. The non-invaded areas were dominated by *Ulex europaeus* L., *Erica vagans* L. and *Armeria pubigera* (Desf.) Boiss. in Punta Nariga, and by *Armeria pubigera* in Sálvora.

In September 2015, for each location, the soil 0–5 and 5–10 cm layers were separately sampled in 10 randomly distributed 15 × 15 cm squares under native vegetation and another 10 under *C. edulis*. Soil subsamples were mixed into a composite sample per site and depth. The soils were sieved (<2 mm), homogenized and kept at 4 °C. The main characteristics of soils are reported elsewhere (Vieites-Blanco and González-Prieto, 2017).

2.2. Soil incubation and gross N transformation rates

Before starting the experiment, soils were wetted to slightly below 70% of their water holding capacity with the wetting system described in Gómez-Rey and González-Prieto (2013), which allows easily wetting highly hydrophobic soils. A paired ^{15}N labelling experiment, with $^{15}NH_4NO_3$ and $NH_4^{15}NO_3$ as tracers and four incubation times (0.5 h, 1, 3 and 7 days), was conducted. Aliquots equivalent to 30 g of dry soil were placed in a total of 192 centrifuge bottles (250 mL): two invaded soils, two non-invaded soils, two soil depths, two ^{15}N-tracers, four incubation times and three replicates. The aliquot of soil corresponding to each bottle was deposited as 4 successive layers, each of which received 1 mL of ^{15}N-tracer solution added uniformly over the soil surface with an automatic pipette (i.e. 4 mL per bottle), equivalent to an N addition of 1 mg kg^{-1} dry soil with a ^{15}N excess fraction of 49%. After labelling, soils were incubated at 25 °C in darkness and, then, an extraction-diffusion method was used for NH_4^+-N and NO_3^--N quantification. Briefly, soils were extracted with 150 mL KCl 2 M (1:5 soil:solution ratio), shaken for 1 h and passed through glass microfibre filters (Whatman GF/A,